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Preparative Separation of Profen Enantiomers by Liquid Chromatography

5º Encontro Nacional de Cromatografia, Sociedade Portuguesa de Química, Universidade de Aveiro, Aveiro, Portugal, 10-12 December, 2007. *(poster)*

P.2 **Preparative separation of profen enantiomers by liquid chromatography**

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Profens, or 2-arylpropionic acids, are an important sub-class of the frequently prescribed non-steroidal anti-inflammatory drugs (NSAIDs). Some of the main primary indications for NSAIDs therapy include rheumatoid arthritis, osteoarthritis, acute gouty arthritis, dysmenorrhea and acute pain. Ketoprofen (R,S)-2-(3-benzoylphenylpropionic acid) and Flurbiprofen (R,S)-2-(2-fluoro-4--biphenylpropionic acid) are examples of NSAIDs, both marketed as racemic mixtures.

It was previously believed that the anti-inflammatory properties of profens reside exclusively in its S-enantiomer, while the R-enantiomer was responsibly for the undesired toxic side effects of these drugs. However, research showed that the R-enantiomers of profens have also a therapeutic action. The R-enantiomer of ketoprofen is now known to have an analgesic and antipyretic action, while the R-enantiomer of flurbiprofen is referred to promote active inhibition on the growth of a variety of human cancer and to slow the progression and pathogenesis of Alzheimer's disease.

In this scenario, the need for the separation of racemic drugs and the consequent use of pure enantiomers has increased and preparative chiral liquid chromatography has become an important separation process for the purification of pharmaceuticals and other added-value products.

The main goals of a chromatographic separation change when moving from an analytical to a preparative scale. Enantioselectivity is commonly the target parameter to be optimized at an analytical scale. However, apart from selectivity, a high loading capacity is an important requisite in preparative separations since this will affect productivity. Additionally, high throughputs in continuous separation processes, such as Simulated Moving Bed (SMB) technology, can be achieved only when high feed concentrations and short cycle times are applied. Thus, it is also necessary a correct selection of the mobile phase composition since it will affect racemate solubility, selectivity and retention times.

PREPARATIVE SEPARATION OF PROFEN ENANTIOMERS BY LIQUID CHROMATOGRAPHY

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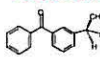
PROFENS AND APPLICATIONS:

Ketoprofen (*R,S*)-2-(3-benzoylphenylpropionic) acid and Flurbiprofen (*R,S*)-2-(2-fluoro-4-biphenylpropionic) acid, belongs to a family of chemicals named 2-arylpropionic acids, or profens, an important sub-class of the frequently prescribed and used drugs called Non-Steroidal Anti-Inflammatory drugs (NSAIDs). Nowadays, preparative chiral chromatography is becoming a more and more important separation process for the purification of pharmaceuticals and other added-value products. Optimization of chiral liquid separations is frequently a complex task that requires, at a preparative scale, a careful selection of its operating conditions. In the case of binary or multicomponent mixtures, an additional complexity results from the competition between the different components for the interaction with the active sites of the stationary phase. The aim of this work is the measurement of adsorption equilibrium data of ketoprofen and flurbiprofen enantiomers, using the adsorption-desorption method, under different mobile phase compositions. Additionally, solubility measurements, pulse and breakthrough experiments of both profens enantiomers on the different mobile phases were performed in order to obtain better understanding of adsorption equilibrium behaviour. Modelling of adsorption data, simulation of fixed-bed and SMB operation were carried out to justify the choice of the mobile phase composition for the preparative separation of ketoprofen and flurbiprofen enantiomers.

NON STEROIDAL ANTI-INFLAMMATORY DRUGS

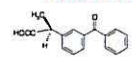
BENEFICTS	RISKS / SIDE EFFECTS
Anti-Inflammatory Rheumatoid Arthritis Osteroearthritis Ankylosing Spondylitis Antipyretic Action Analgesic Action Dismenorrea	Gastrointestinal toxicity Hepatic toxicity Renal toxicity Cardiovascular failure

S-KETOPROFEN



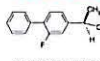
Anti-inflammatory Analgesic

R-KETOPROFEN



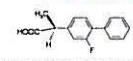
Periodontal diseases prevention Analgesic and Antipyretic

S-FLURBIPROFEN



Anti-inflammatory Analgesic

R-FLURBIPROFEN

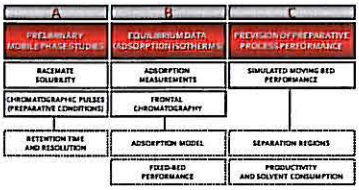


Anti-cancerinogenic, Analgesic *Slow progression of Alzheimer's?*

EQUIPMENT AND METODOLOGY:

CHIRAL LIQUID CHROMATOGRAPHY

1. Jasco HPLC system UV-1575 detector with a preparative cell (1.0mm).
2. A Manual Rheodyne 7725(i) injection valve with three different loops: 20µL, 100µL and 1mL.
3. Eldex controlled column oven.
4. Two HPLC columns (Chiralpack AD) with different particle sizes: 10 µm (analytic) and 20 µm (preparative).
5. A thermostatic water bath.



SOLUBILITY: Gravimetric method

$$S = 10^3 \frac{(m_{sa} - m_s)}{(m_{sa} - m_{sa})} \quad \text{(g profen/kg solvent)}$$

ADSORPTION ISOTHERMS: Saturation-Regeneration method

$$C_i^f V^a = \varepsilon V_c C_i^f + (1-\varepsilon) V_c q_i^*$$

MODELLING: Binary Competitive Adsorption Isotherms

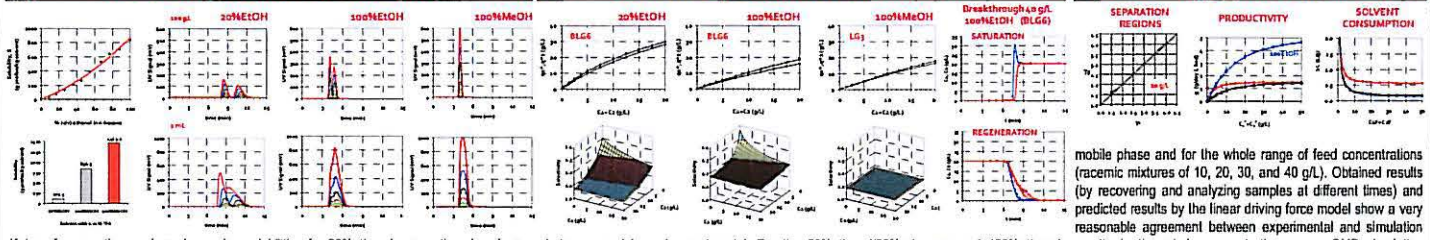
Langmuir (LG)	$q_i^* = \frac{Q_i b_i C_i}{1 + b_1 C_1 + b_2 C_2}$
Linear + Langmuir (LLG5)	$q_i^* = m_i C_i + \frac{Q_i b_i C_i}{1 + b_1 C_1 + b_2 C_2}$
bi-Langmuir (BLG6)	$q_i^* = \frac{Q_{1i} b_{1i} C_i}{1 + b_1 C_1 + b_2 C_2} + \frac{Q_{2i} b_{2i} C_i}{1 + b_1 C_1 + b_2 C_2}$

KETOPROFEN ENANTIOSEPARATION:

A SOLUBILITY AND ELUTION CHROMATOGRAPHY

B COMPETITIVE ADSORPTION EQUILIBRIUM ISOTHERMS

C PERFORMANCE AT PREPARATIVE SCALE (SMB)



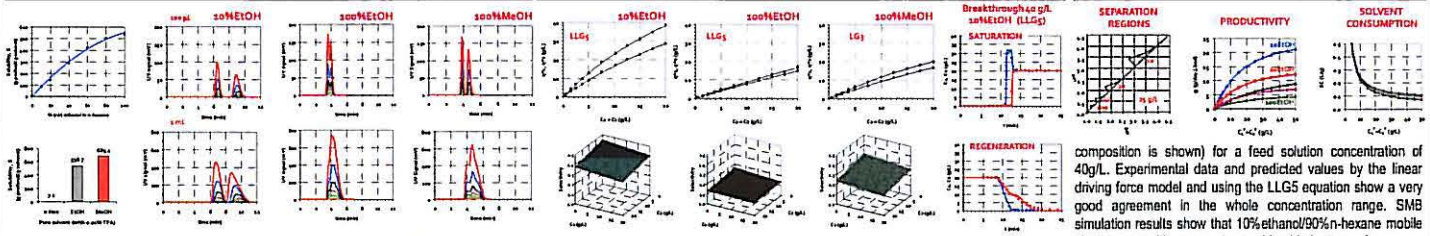
Ketoprofen enantiomers have increasing solubilities for 20%ethanol, pure ethanol and pure methanol. Ketoprofen is insoluble in pure n-hexane and present increasing solubilities with the increase of the ethanol content. The increase of the amount injected leads to a decrease in the retention time of both enantiomers. The hydrocarbon mobile phase: 20%ethanol/80%n-hexane presents considerable higher retention times than the pure mobile phases (ethanol and methanol). Despite higher ketoprofen solubility, pure methanol does not allow acceptable selectivity values and, consequently, ketoprofen enantioseparation. Using a Levenberg-Marquardt algorithm for the adsorption isotherms modelling, it's obtained a good agreement between models and experimental. For the 20%ethanol/80%n-hexane and 100%ethanol mobile phases, a bi-Langmuir isotherm better simulate the experimental data obtained. Adsorption behaviour with 100%methanol mobile phase is well described by the Langmuir model. For 100%methanol, selectivity is low and constant. For 20%ethanol/80%n-hexane mobile phase, despite its high selectivity for low concentrations, presents a strong decrease in selectivity with the increase of concentrations. The better situation is obtained for 100%ethanol, where selectivity maintains high values even for high enantiomer concentrations. Different saturation and regeneration curves were carried out for pure ethanol mobile phase and for the whole range of feed concentrations (racemic mixtures of 10, 20, 30, and 40 g/L). Obtained results (by recovering and analyzing samples at different times) and predicted results by the linear driving force model show a very reasonable agreement between experimental and simulation results in the whole concentration range. SMB simulation results show that pure ethanol presents considerable better performances (bigger separation regions). At high feed concentrations (for example, 40 g/L of racemic mixture) the productivity using pure ethanol is 3 times the obtained with the other two solvents; the correspondent solvent consumption is only 75% and 25% of the one needed with pure methanol and 20%ethanol/80%n-hexane, respectively.

FLURBIPROFEN ENANTIOSEPARATION:

A SOLUBILITY AND ELUTION CHROMATOGRAPHY

B COMPETITIVE ADSORPTION EQUILIBRIUM ISOTHERMS

C PERFORMANCE AT PREPARATIVE SCALE (SMB)



Solubility results obtained for flurbiprofen were similar of the ones obtained for ketoprofen. Flurbiprofen solubility values increase for 100%n-hexane, 100%ethanol and 100%methanol. A significant higher solubility value is obtained using a mobile phase based on high polar content and retention times decrease with the increasing of alcoholic content. However, better selectivities are obtained for low alcoholic content. The hydrocarbon/methanol-based solvent was not used due to its immiscibility above 10%methanol in n-hexane. A 10%ethanol/90%n-hexane composition represents a reasonable compromise between selectivity, retention time and solubility. A good agreement was obtained between experimental data and proposed adsorption isotherms models. Although Langmuir and linear+Langmuir models describe reasonably adsorption behaviour on the ethanol/n-hexane mobile phase composition, a more complex model, such as the modified linear+Langmuir isotherm (different *m* values), simulates better the experimental data obtained. For the 100%methanol mobile phase, adsorption behaviour is also better described by the modified linear+Langmuir model. Results show a decrease in selectivity with the increase of alcoholic content. The best situation is obtained for a 10%ethanol/90%n-hexane solvent composition. Several ethanol/n-hexane-based mobile phase compositions were tested in breakthrough experiments (only 10/90 composition is shown) for a feed solution concentration of 40g/L. Experimental data and predicted values by the linear driving force model and using the LLG5 equation show a very good agreement in the whole concentration range. SMB simulation results show that 10%ethanol/90%n-hexane mobile phase composition presents considerable better performances (bigger separation regions). Under preparative conditions, maximum productivity is obtained with 10%ethanol/90%n-hexane due to higher selectivity. Solvent consumption is not significantly different between the four mobile phase compositions.

ACKNOWLEDGEMENTS:

Financial support by the Portuguese R&D Foundation FCT and European Community FEDER (projects POCTI/EQU/38811/2001 & POCTI/EQU/59738/2004).

The authors wish to thank Simão P. Pinho (IPB) for the support on the solubility measurements.

CONCLUSIONS:

Results show that, for ketoprofen enantioseparation, pure ethanol is a better mobile phase than the usual high alkane content mobile phases: it allows higher solubility of the racemate, lower retention times and also higher selectivity at high enantiomer concentrations.

For the flurbiprofen enantioseparation, a 10%ethanol/90%n-hexane mobile phase composition is proposed. In spite of lower solubility (when compared with a higher polar content mobile phase), high selectivity values are obtained under preparative conditions and within an acceptable retention time.

These results become evident that individual studies must be carried out for each enantioseparation system, since different profen drugs may have different behaviours.